"On the Optical Activity of the Nucleic Acid of the Thymus Gland." By Arthur Gamgee, M.D., LL.D., F.R.S., Emeritus Professor of Physiology in the Owens College, Victoria University, and Walter Jones, Ph.D., Associate Professor of Physiological Chemistry in the Johns Hopkins University. Received May 15,—Read May 28, 1903.

We have lately shown* the dextrorotatory character of the nucleoproteids of the pancreas, thymus, and suprarenal gland. We have, in the course of our investigations, shown that the "nucleins" possess a stronger rotation than the "nucleoproteids" properly so called, and from which they are derived, and in the researches which we have planned, and which naturally are suggested by our previous work, the first step appeared to us to be to determine the optical activity of the nucleic acids corresponding to the nucleoproteids investigated by us.

In the present paper we shall confine our attention to the optical activity of thymus-nucleic acid, prepared by the method of Kossel and Neumann.† We adhered closely to the method recommended by these chemists, which furnishes with great ease a colourless product, yielding colourless and perfectly transparent solutions admirably adapted for polarimetric observations. From 6 kilogrammes of trimmed thymus Kossel and Neumann obtained 120 grammes of pure nucleic acid. From 600 grammes of the gland we obtained 9·5 grammes, though no special pains were taken to work even in an approximately quantitative manner. Our purified product, like that of Kossel and Neumann, was free from proteid and barium.

1. An amount of nucleic acid weighing 1·109 grammes was suspended in water and dissolved by the cautious addition of dilute solution of ammonia in small quantities, so that when solution had been effected the reaction of the solution towards litmus was neutral, The volume of the solution was made up to 100 c.c. and it was then examined polarimetrically.

Weight of substance (W)...............	1·109 grammes.
Volume of solution (V)	100 c.c.
Length of tube (L)	200 m.m.
Observed angle (α)	$+3^\circ\ 29'$.

$$[\alpha]_D = +156^\circ{\cdot}9.$$

2. 10 c.c. of the above neutral solution were diluted with 10 c.c.

* Gamgee and Jones, "On the Nucleoproteids of the Pancreas, Thymus, and Suprarenal Gland, with especial Reference to their Optical Activity," 'Roy. Soc. Proc.,' vol. 71 (1903), p. 385.

† Kossel and Neumann, 'Ber. d. Deutsch. Chem. Ges.,' vol. 27, p. 2215.

of distilled water and the resulting solution was examined polarimetrically.

$$W = 1{\cdot}109 \text{ grammes}; \; V = 200 \text{ c.c.}; \; L = 200 \text{ m.m.}; \; \alpha = 1^\circ \, 43'.$$
$$[\alpha]_D = +154^\circ{\cdot}2.$$

3. 10 c.c. of the solution used in the last experiment were further diluted with 10 c.c. of distilled water and the resulting solution was examined.

$$W = 1{\cdot}109 \text{ grammes}; \; V = 400 \text{ c.c.}; \; L = 200 \text{ mm.}; \; \alpha = +0^\circ \, 52'.$$
$$[\alpha]_D = +156^\circ{\cdot}4'.$$

The above observations indicate that solutions of the nucleic acid of the thymus are powerfully dextro-rotatory, but that the specific rotation of neutral solutions does not vary appreciably with dilution, the variations in the results of the three sets of observations recorded above falling within the probable limits of error. Similar results had already been obtained with solutions of the nucleoproteid of the pancreas, although the limits of dilution in that case were not so great as in the present instance.

On the Influence of the Reaction of the Solution on the Optical Activity of the Nucleic Acid of the Thymus Gland.

The observations about to be referred to indicate a very remarkable influence exerted by the reaction of the solution on the optical activity of the nucleic acid under discussion. The rotation is notably influenced by the acidity of the solution; it reaches a maximum at a certain degree of acidity and then decreases. On the other hand, the addition of ammonia in sufficient proportion will render a solution of thymus-nucleic acid optically inactive, though neutralisation of the acid will restore its pristine activity. It is to be noted, however, that there is no abrupt change around the neutral point, a statement which is illustrated by the fact that two solutions of equal concentration may be prepared which are undistinguishable in so far as their optical rotation is concerned, one of which is faintly, though distinctly, acid to litmus, while the other is alkaline.

4. 25 c.c. of the original neutral solution of nucleic acid employed in the first set of experiments were made decidedly acid by the addition of a trace of 20 per cent. acetic acid. The change of volume was so small as to be negligible. The solution was then subjected to polarimetric examination with the following results:—

$$W = 1{\cdot}109 \text{ grammes}; \; V = 100 \text{ c.c.}; \; L = 200 \text{ mm.}; \; \alpha = +3^\circ \, 30'.$$
$$[\alpha]_D = +157^\circ{\cdot}8.$$

5. 25 c.c. of the original solution were correspondingly made distinctly alkaline with ammonia and polarimetrically examined :—

$$W = 1{\cdot}109 \text{ grammes}; \; V = 100 \text{ c.c.}; \; L = 200 \text{ mm.}; \; \alpha = +3^\circ\ 31'.$$

$$[\alpha]_D = +158^\circ{\cdot}7.$$

The two above sets of experiments show that determinations of the optical activity of thymus-nucleic acid may be made in a neutral or quasi-neutral fluid, without fear of variation caused by inaccuracy in observing the point of exact neutrality. The following experiments, however, demonstrate that the rotation is markedly changed by the addition to a neutral solution of thymo-nucleic acid either of a considerable amount of acetic acid or of ammonia.

6. 20 c.c. of the original solution were treated with one-tenth of a c.c. of 20 per cent. acetic acid and the solution was polarimetrically examined :

$$W = 1{\cdot}109 \text{ grammes}; \; V = 100{\cdot}5 \text{ c.c.}; \; L = 200 \text{ mm.}; \; \alpha = +3^\circ\ 33'.$$

$$[\alpha]_D = +160^\circ{\cdot}4.$$

7. 10 c.c. of the solution used in 6 were treated with 0·3 c.c. of 20 per cent. acetic acid and polarimetrically examined :

$$W = 1{\cdot}109 \text{ grammes}; \; V = 101{\cdot}5 \text{ c.c.}; \; L = 200 \text{ mm.}; \; \alpha = +3^\circ\ 36'.$$

$$[\alpha_D] = +164^\circ{\cdot}7.$$

Up to this point we observe an increase in the specific rotation of the nucleic acid. If, however, as in the two following experiments, the amount of acetic acid is increased, the optical rotation undergoes diminution.

8. 10 c.c. of the original solution were treated with 1 c.c. of 20 per cent. acetic acid and then polarimetrically examined :

$$W = 1{\cdot}109 \text{ grammes}; \; V = 110 \text{ c.c.}; \; L = 200 \text{ mm.}; \; \alpha = +2^\circ\ 29'.$$

$$[\alpha]_D = +123^\circ.$$

The remarkable influence of solution of ammonia is illustrated by the following observations :—

9. 20 c.c. of the solution which had been used in observation 5 were treated with 0·5 c.c. of 10 per cent. solution of ammonia and polarimetrically examined :

$$W = 1{\cdot}109 \text{ grammes}; \; V = 102{\cdot}5 \text{ c.c.}; \; L = 200 \text{ mm.}; \; \alpha = +2^\circ\ 40'.$$

$$[\alpha]_D = +123^\circ{\cdot}4.$$

10. The solution used in experiment 9 was treated with an equal volume of 10 per cent. ammonia and the resulting solution examined :

$$W = 1{\cdot}109 \text{ grammes}; \; V = 205 \text{ c.c.}; \; L = 200 \text{ mm.}; \; \alpha = +0^\circ\ 35'.$$

$$[\alpha]_D = +50^\circ{\cdot}8.$$

11. The solution used in experiment 10 was further diluted with an equal volume of 10 per cent. ammonia, when the solution was found to be optically inactive.

Our observations have shown us, as has been mentioned at an earlier part of this paper, that the diminution or abolition of optical activity which is induced by alkalies in solutions of thymo-nucleic acid are not permanent, the addition of acid restoring the primitive optical condition.

Bülow has shown* that the optical rotation of proteids varies with changes in the reaction of their solutions, and Framm† studied especially the alteration brought about in the specific rotation of gelatine by the addition of acids or alkalies. The alterations observed by Framm, however, were probably due to fundamental chemical changes brought about by the acid or alkali on the proteid. This reasoning cannot, however, be applied to the case of the nucleic acid of the thymus gland, seeing that the addition of acid to an alkaline solution restores the optical activity.

At the suggestion of one of us (A. G.), Dr. Thomas B. Osborne‡ has determined the optical activity of the nucleic acid which he separated from the wheat embryo, and to which he applied the name of tritico-nucleic acid. Dr. Osborne§ has found that this nucleic acid is dextrorotatory, though the degree of rotation is considerably influenced by the concentration of the solution. A solution containing 4 per cent. of tritico-nucleic acid possessed a specific rotation $[\alpha]_D = +73°$.

* Bülow, "Ueber aschefreies Eiweiss," 'Pflüger's Archiv,' vol. 58 (1894), p. 207.

† Framm, "Untersuchungen über die specifische Drehung des β-Glutin," *ibid.*, vol. 68 (1897), p. 144.

‡ T. B. Osborne and I. F. Harris, "Die Nucleinsäure des Weizenembryos," 'Zeitschr. f. Physiol. Chemie,' vol. 36, heft 2 (September, 1902), p. 85.

§ T. B. Osborne, "The Specific Rotation of the Nucleic Acid of the Wheat Embryo," 'Amer. Journ. of Physiol.,' vol. 9, No. 2 (issued April 1, 1903), p. 69.